CROPS RESEARCH

ARS-34-25
JULY 1961

A Rapid Method for the Determination of WATER-SOLUBLE REDUCING SUBSTANCES IN RAW COTTON FIBER

Agricultural Research Service
U. S. DEPARTMENT OF AGRICULTURE

CONTENTS

Growth Through Agricultural Progress

A RAPID METHOD FOR THE DETERMINATION OF WATER-SOLUBLE REDUCING SUBSTANCES IN RAW COTTON FIBER

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ABSTRACT

A method for practical routine use in measuring the amount of water-soluble reducing materials in raw cotton fiber is described. The procedure is derived from the dinitrosalicylic acid method of Sumner (8)[2] for estimation of sugars in urine. It is called the DNS test in reference to the reagent used and its result is designated a DNS value, numerically identical with the reducing value in terms of percent glucose.

Noteworthy advantages of the DNS test are its speed and simplicity, the stability of the original reagent, and the color stability of the reaction product. The test is adapted to use with a 100-mg. or a 1-g. sample. Of the two modifications of the method, the analysis with the 1 g. sample is the more rapid and appears to be better adapted to most types of routine testing.

Important features of the methodology of the DNS test are reported. In addition, certain data are presented to illustrate the use of the test and the range of values found in commercial raw cotton. Within the United States Cotton Belt, the highest values have been noted in cotton produced in the Texas-Oklahoma and Western areas.

BACKGROUND

The need for new and improved methods of testing the properties of raw cotton fiber has been emphasized repeatedly in recent years by persons concerned with cotton production, marketing, and utilization. To be generally acceptable, a method must be simple, safe, relatively inexpensive, at least moderately precise, and, above all, rapid. The present contribution represents an attempt to supply a test which fulfills these requirements.

Certain earlier methods similar in purpose to the one here described have involved copper reduction techniques, but these methods should be distinguished from "copper number" procedures. Copper reduction procedures have been applied to chemically processed cotton yarns and fabrics by many workers. The test result, referred to as the "copper number" of the test item, has been interpreted as a measure of degradation of the cellulose of the fiber (7). When such procedures are used, however, with raw cotton, the reduction that occurs is partially a result of a reaction between the copper and normally present water-soluble constituents in the undamaged fiber, probably principally reducing sugars. Fargher (1) used such a method in the latter manner. Under the conditions employed in a copper reduction procedure described from this laboratory (6), the reaction is mainly a reflection of the amount of water-soluble reducing materials present. Hall and Elting (3) extracted raw cotton with hot water and tested the extract with a commercial tablet of a copper-reduction type used medically to estimate reducing sugars in urine. Their method (3) has been employed by the Agricultural Marketing Service in testing commercial cottons (9, 10, 11, 12).

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[2] Figures in parentheses refer to Literature Cited at end of this publication.

According to at least three reports (1, 3, 6), reducing values of raw cotton are related to economically significant properties of the fiber. The tendency of the fiber to turn brown, either in long-term storage or during short exposure at high temperature, has been shown to be approximately proportional to the copper reduction value (6). Fargher (1) indicated that high copper reduction results are accompanied by a high capacity of the fiber for mildewing in humid storage. Hall and Elting (3) have suggested that low values in raw cotton indicate that microorganisms have already grown on the fiber, also that abnormally high ones in some cases are indicative of cotton contaminated with aphid honeydew.

The writers believe that the previously available methods for determining the content of water-soluble reducing materials in raw cotton fiber have not been entirely adequate for routine use. A copper reduction method described earlier (6), although quite precise, is also somewhat time consuming. A rapid method has been reported in which picric acid is used (6), but the DNS method as here detailed is considered to be more rapid and simpler than any previous procedure. The method for the 1-g. sample requires less time than that for the 100-mg. sample.

MATERIALS AND APPARATUS

1. DNS reagent--to 300 ml. of 4.5 percent carbonate-free NaOH, add 880 ml. of 1 percent dinitrosalicylic acid, (3,5-$(NO_2)_2C_6H_2$-2-OHCOOH) and 255 gs. of Rochelle salt (sodium potassium tartrate, $NaKC_4H_4O_6.4H_2O$). Mix until dissolved and keep tightly stoppered.

2. Aerosol OT, solid (dioctyl sodium sulfosuccinate). Prepare a 0.02 percent solution in distilled water for extracting samples.

3. Extraction tubes. These consist of pieces of glass tubing 16 cm. long and of approximately 4 mm. inside diameter.

4. One straight 10-inch piece of heavy wire, or other similar suitable device for forcing samples into extraction tubes.

5. Metal washers, 8 mm. inside diameter and 19 mm. outside diameter, that is, $\frac{1}{4}$-inch flat washers. These are used with small rubber bands to hold extraction tubes in position in the colorimeter tubes during extraction.

6. Optically matched 15 ml. colorimeter test tubes of 14 mm. outside diameter, marked at 10 ml.

7. Support, test tube, Army Medical School model, stainless steel, 6-$\frac{1}{4}$ inches long, 2 inches wide, with 12 openings for tubes up to 15 mm. in diameter. The number of such supports needed depends on the number of samples being analyzed.

8. Pipette, 2 ml., graduated, with long tip; a Luer-Lok syringe (2 ml.)--or a mechanical pipettor (Cornwall) which can be set for a predetermined volume, may be advantageously substituted if many samples are to be tested.

9. Pipette--1 ml., graduated, serological.

10. Flat-bottomed Pyrex baking dish, 8 x 4 inches on bottom surface, 2-$\frac{1}{2}$ inches deep, or other suitable Pyrex or metal vessel for holding tubes in boiling water during color development.

11. Electric hot plate, 8 inches in diameter, or other suitable heating device.

12. Polyethylene washing bottle, 250 ml. capacity.

13. Colorimeter, photoelectric, Klett-Summerson, Model 900-3 or other suitable colorimeter.

DESCRIPTION OF STANDARD PROCEDURES

A. Preparation of color standards

For either the colorimetric methods or the visual comparison methods as described under B, C, and D below, it is necessary to prepare a color standard. This may be done as follows.

1. Weigh 100 mg. of glucose into a 100 ml. volumetric flask and add distilled water, containing 0.02 percent Aerosol OT, to the mark. Into a series of 11 matched 15 ml. colorimeter tubes in a test tube support, pipette a graded series of different amounts of this glucose solution, i.e. 0, 0.1 ml., 0.2 ml., 0.3 ml.-----1.0 ml. Add appropriate amounts of distilled water to bring the volume in each tube to 1.0 ml., i.e. 1.0 ml; 0.9 ml., 0.8 ml., 0.7 ml.-----0 ml. This series of tubes then contains 0 to 1.0 mg. (0 to 1,000 μg) of glucose.

2. Heat tubes, in test tube support, in boiling water for 5 minutes. Add 1 ml. of DNS reagent to each tube, place tubes back into boiling water bath for 10 minutes, remove from bath, and cool in running water for at least 5 minutes. Dilute the solution in each tube to the 10 ml. mark with water by use of the polyethylene washing bottle, set the colorimeter at its null point with the "zero glucose" sample, and make readings to provide a standard curve of colorimeter reading versus DNS value. Use a green 500 mμ filter (No. 50), as supplied with the Klett-Summerson[3] colorimeter. In this curve, 0.1 mg. or 100 μg glucose corresponds to a DNS value of 0.1, etc. for either the 100 mg. sample or 1-g. sample of cotton fiber.

For laboratories making repeated use of the visual comparison method over a period of months permanent color standards may be useful. These can be prepared according to the general procedure described by Sumner (8). For the cotton analysis this is carried out as follows. Make a 0.8625 percent solution in water from clear crystals (avoid old samples) of ferric ammonium sulfate ($Fe\ NH_4(SO_4)_2.12H_2O$) and a 1-percent solution of dinitrosalicylic acid; pipette into a series of 11 matched 15 ml. colorimeter tubes a graded series of amounts of the ferric ammonium sulfate solution, i.e. 0, 0.07 ml., 0.14 ml., 0.21 ml.-----0.70 ml. and then add 1-percent dinitrosalicylic acid to the 10 ml. mark. These solutions then correspond in visual color to DNS values of 0, 0.1, 0.2, 0.3-----1.0. Comparisons are made by observation down through the solution in the tubes.

B. Fiber analysis, colorimeter method, 100-mg. sample

1. Weigh out 100-mg. samples of the raw cotton to be tested, each sample being made up of at least 5 separate pinches of fiber. Force each sample up into an extraction tube with a stiff wire, the fiber being packed into a tight cylinder of approximately 20 mm. in length, with the bottom of the sample plug about 5 to 10 mm. above the lower end of the tube. Place each extraction tube, containing sample, into position in a 15 ml. colorimeter tube, using a washer and a rubber band wrapped several times around the extraction tube to elevate it so that the outlet end is about 50 mm. above the bottom of the colorimeter tube. By use of a 2 ml. graduated pipette with a long tip or a graduated syringe, add 1.3 ml. of water containing 0.02 percent Aerosol OT at the top of the extraction tube. Allow about 20 to 30 minutes for passage of water through the fiber sample, at which time about 1 ml. of liquid should have collected in the tube. If an air bubble should appear in the extraction tube, this may be released with a small glass capillary (e.g. melting point tube).

2. Carry out color development and measurement as described under A 2 above, setting the colorimeter to the null point with a zero-glucose tube.

3. Read the DNS value from the standard curve as prepared under A 1 and A 2 above.

[3] Use of a product name does not imply recommendation by the U. S. Department of Agriculture to the exclusion of other products which may also be available.

C. Fiber analysis, colorimeter method, 1-g. sample

1. Weigh out 1-g. samples of the raw cotton to be tested, each sample being made up of at least 5 separate pinches of fiber. Place each sample into a 50 ml. beaker and add 10 ml. of water containing 0.02 percent Aerosol OT. Tamp the fiber thoroughly in the water with a glass rod and knead it for several seconds. With a pipette withdraw 1 ml. of liquid from the beaker and place it into a 15 ml. colorimeter tube.

2. Proceed with completion of analysis as described under A 2 and B 3 above.

D. Fiber analysis, visual comparison method, 100-mg. sample or 1-g. sample

Carry out extraction and color development as described under B or C above. Then compare color in the experimental tubes visually with that in the glucose color standard tubes or permanent standard tubes prepared as described under A above and estimate the DNS value. Color is judged by looking down through the solutions in the tubes.

METHODOLOGY

The DNS method was developed originally for a 100-mg. sample and was later adapted to a 1-g. sample. Except as is otherwise indicated, the results here reported involved use of the 100-mg. method. Several features of the methodology as described in relation to the 100-mg. sample obviously relate equally to the procedure with the larger sample.

Method for 100-mg. Sample

Completeness of extraction

In order to test the completeness of extraction of water-soluble reducing materials in the 100-mg. method, certain samples were analyzed by the procedure indicated (B, above) and then the same cotton plugs were left in the extraction tubes, extracted again with a second 1.3 ml. portion of water, and the resulting second extracts analyzed. This procedure was applied to two cotton samples, one of a moderate reducing level and the other of a high level; it was replicated four times for each of the two samples. The results on the first extraction of the first sample were 0.35, 0.37, 0.30, and 0.36; on the second extraction of the same sample, values of 0.02, 0.03, 0.00, and 0.03 were obtained. On the first extraction of the second sample, values of 0.80, 0.90, 0.81, and 0.80 were found; on the second extraction of the same samples, the values were 0.00, 0.00, 0.01, and 0.00. Obviously the first extraction was essentially complete.

A further study was then made in an effort to determine approximately at what point in the extraction process the greater part of the reducing substances have been removed from the sample. With the extraction tubes employed, 1 ml. of extract consisted on the average of 30 drops. Accordingly, several extractions were made in which successive 5-drop (0.17 ml.) increments were collected and DNS determinations carried out on each increment. With a sample having a total DNS value by the standard procedure of 0.25, values of 0.20, 0.05, 0.00, and 0.00 were obtained in tests on four successive 5-drop amounts of extract. With a sample of total DNS value 0.75, the corresponding values were 0.60, 0.11, 0.04, and 0.00. It is evident that on both types of sample the greater portion of the water extractable reducing substances is extracted in the first increment (5 drops) and that no more can be detected by this method after 1/2 ml. (15 drops). The remainder of the extracting liquid acts then as an extra margin of safety against any variation in the efficiency of the extraction.

Temperature and time for color development

When the test as here specified was altered by changing the temperature of the water bath used during the development of color, it was observed that variation in the bath temperature, at least in the range of 80 to 100 degrees C., had very little if any measurable effect on the depth of color produced.

When the time of color development was varied from 5 to 15 minutes, using a bath temperature of 100 degrees C., the colorimeter readings exhibited essentially no change. A 10-minute period and a 100-degree temperature have been selected for use in the standard procedure. Thus, neither the exact temperature nor time of color development is critical in the range selected for use in the standard DNS procedure. It is of importance that after color development the heated tubes should be cooled. A slight orange coloration develops in the zero-glucose blank during the boiling process; this color disappears after 5 minutes in cold running water.

Stability of developed color with time

The color produced in glucose standard tubes or in fiber extracts has proven to be very stable in the sense that colorimeter readings taken up to 8 days after color development have remained essentially unchanged. Tubes that are to serve as comparison standards for several days should, however, be sealed with paraffined corks to prevent volume changes by evaporation. It is recommended that fresh standards be prepared occasionally, particularly when a fresh reagent is being used.

Recovery of glucose added to fiber extract

As a further check on the analytical procedure, known quantities of glucose were added to fiber extracts and the efficiency of recovery determined by comparing the actual DNS values of such samples with values predicted on the basis of the DNS value of the original extract and the amount of added glucose. Any marked deviation from the predicted values would suggest the presence of some unknown substance or substances in the extract which altered the reaction of glucose with the reagent. Six 100-mg. samples of greenhouse-grown cotton were extracted and the extracts combined and mixed. Two 1-ml. aliquots of the extracts were employed as controls. Three additional aliquots of the extract were added to colorimeter tubes containing 100, 200, and 300 micrograms respectively, of glucose. Glucose in 80 percent ethanol had been added to clean dry colorimeter tubes and the alcohol evaporated to near dryness before introduction of the cotton extract. DNS values were determined on the control extracts and on those containing added glucose. The added glucose was recovered exactly in each of the three added levels.

Malic acid, the predominant organic acid found in unweathered cotton fiber (2), did not exhibit a color reaction with the reagent, nor did it influence the color reaction with glucose, even when added in amounts equal to that of the glucose present. The same result was obtained with citric acid, the next most abundant acid in the fiber. The DNS reagent has been found to give a proportional color reaction with fructose and cellobiose as well as with glucose. No reaction was obtained with sucrose or raffinose.

Reproducibility of results

In practical situations with commercial cotton the reproducibility of results in the DNS test seems likely to be affected by the thoroughness of measures taken to reduce sampling error. The 100-mg. sample should be made up of at least five small pinches of fiber from the parent sample in order to reduce this error. The source of the problem may be seen in the fact that variation in DNS values on fiber from one boll to the next may be high. This situation is illustrated by the following data on fiber from 9 individual bolls picked on the day of opening from plants within about 100 feet of uniform row of a single cotton variety (Coker 100, Florence, S. C., crop of 1960): 0.53, 0.23, 0.22, 0.18, 0.69, 0.32, 0.14, 0.33, and 0.14. A further factor of probable importance is the difference in DNS values between fibers from the chalazal or rounded end of the seed and those obtained on fibers originating from other parts of the seed. In one case chalazal fibers exhibited a DNS value of 0.26, whereas nonchalazal fibers from the same seeds had a value of 0.11.

Sensitivity and linearity of color response

There is a tendency for lower sensitivity and a departure from linearity in standard curves at very low glucose concentrations (100 micrograms or below). Sumner (8) noted this decreased sensitivity at such concentrations and was of the opinion that it was due to the destruction of reducing substances by dissolved oxygen in the solution. He obtained a more satisfactory color development at low levels after adding Rochelle salt to the reagent to prevent it from dissolving oxygen. The present investigators attempted to further diminish any effects of dissolved oxygen by heating the glucose standard solutions and fiber extracts 5 minutes in boiling water to drive off oxygen prior to addition of the DNS reagent. It is found that in general this preheating results in a measurable deeper color formation. The color development can be shown to be sensitive to the presence of oxygen by bubbling this gas through the solution during the color development process; a much decreased color formation occurs when this is done.

Determinations on tinged samples

A question occurred about the extent to which the water-extractable brownish coloring matter found in some cottons might interfere with the DNS test. Two deeply tinged commercial samples were extracted, DNS reagent added, and then colorimeter readings taken without heating. The amount of color present from the extract was just measurable, was equivalent to approximately 0.03 to 0.05 DNS units in the two cases.

In order to determine the general level of DNS values to be expected from tinged cotton, several determinations were run on 1960 crop samples in this category. Two samples classified as Middling Tinged were analyzed for each of eight locations. They had DNS values as follows: Altus, Okla.,--0.41, 0.34; Atlanta, Ga.,--0.16, 0.16; Birmingham, Ala.,--0.14, 0.14; Columbia, S. C.,--0.38, 0.68; Lubbock, Tex.,--0.84, 0.53; Oklahoma City, Okla.,--0.16, 0.24; Raleigh, N. C.,--0.14, 0.17. It may be seen that although there were a few higher-than-average values in this group of samples, several low values also occurred.

Determination on thin-walled fiber

Thin-walled cotton fiber is generally considered to swell more in water than thick-walled fiber. It was thought possible that for this reason, as well as because of its high surface per unit weight, thin-walled cotton samples might seriously impede the flow of water in the extraction procedure as here described. To test this possibility 6 samples of fiber were obtained from a potassium deficiency fertilizer plot at Florence, S. C., and tested. These samples, ranging in arealometer value from 374 to 524, were all extracted completely in 20 to 40 minutes. The time of extraction is apparently more related to the degree of packing of the sample in the extraction tube than to the wall thickness of the fiber, at least within the range of wall thickness tested.

Method for 1-g. Sample

Subsequent to experiments with 100 mg. samples it was found possible to adapt the method also to 1-g. samples. By simply tamping 1 g. of fiber in 10 ml. of water containing 0.02 percent Aerosol OT and then analyzing 1 ml. of the liquid, results similar to those with the 100 mg. sample could be obtained. For example, samples analyzed by the 100 mg. method and the 1-g. method gave results as shown in Table 1. There appears to be no reason to believe that reducing substances are adsorbed to any material extent on the fiber in the 1-g. method. This conclusion is derived from the data of Table 1 and has also been shown in another experiment. A well mixed sample of fiber was analyzed by the 100-mg. method, yielded a DNS value of 0.45. Then it was analyzed by the 1-g. method but the fiber was kneaded with 10 ml. of solution containing 200 μg. per ml. of glucose. The DNS value as determined on the liquid was exactly 0.65.

The information on various features of color development and measurement as reported for the 100-mg. method in the sections above obviously applies equally to the 1-g. method. The 1-g. method is more rapid than the 100-mg. method. An additional advantage of the

TABLE 1.--Comparative DNS values on 10 cotton fiber samples by the 100-mg. method and the 1-g. method

Sample	DNS value*		Sample	DNS value*	
	100-mg. method	1-g. method		100-mg. method	1-g. method
Winnsboro #118.....	0.11	0.14	El Paso #76.....	0.52	0.64
Florence, 1960.....	.17	.14	El Paso #94.....	.58	.58
Augusta #13.....	.32	.28	El Paso #101.....	.72	.69
Fresno #95.....	.37	.40	Atlanta #27.....	.81	.91
Corpus Christi #50...	.39	.37	Calcot, 1960.....	1.03	.91

*Averages of triplicate determinations.

1-g. method is that the aqueous-extract pH of the fiber may be determined on the liquid from the sample remaining after removal of the 1 ml. for DNS analysis. If such a combination procedure is employed, the extracting water should be boiled and cooled before use. Either the Fisher Universal pH indicator or a glass electrode pH meter have been found suitable in measuring the pH.

SOME ILLUSTRATIVE DATA FROM ANALYSES

It has been established that copper reduction values decrease when cotton is subjected to humid preharvest weathering and that these decreases are accompanied by increases in aqueous-extract pH (5, 6). Similarly, DNS values also decrease; data illustrating this point may be seen in Table 2.

Earlier data have shown that raw cotton incubated in a humid atmosphere with fungi in pure culture undergoes an increase in aqueous-extract pH and a decrease in copper reduction value (4). It might be assumed that the DNS value would also decrease during such incubation. An experiment proved the correctness of the assumption. Raw cotton was incubated in a water vapor saturated atmosphere with Fusarium moniliforme 1004.1 according to a procedure previously described (4). At incubation intervals of 0, 2, 3, 4, 7, 9, and 11 days the aqueous-extract pH values were 6.5, 7.0, 7.2, 9.4, 10.0, 10.0, and 10.0 and the DNS values were 0.27, 0.28, 0.28, 0.13, 0.05, 0.07, and 0.07.

In order to try out the DNS method on a range of types of commercial cotton, 144 samples from the United States crop of 1959 were tested, these samples being of known grade and ginning area. The results are shown in Table 3. It may be noted that DNS values equal to or greater than 0.30 were found exclusively in cotton from the Texas-Oklahoma and Western areas. This trend for localization of cotton of high reducing value in western regions has been noted also in a series of 400 graded samples analyzed by an earlier copper reduction procedure (6), and is seen in Agricultural Marketing Service data (9, 10, 11, 12). A trend for lower DNS values to be found at lower grade levels in the Southeast is also seen in Table 3.

Earlier data have indicated that raw cotton of high copper reduction value tends to turn brown more than cotton of lower value, both in storage and at an elevated temperature (6). The browning tendency of cotton might be assumed therefore to be related also to the DNS value. This, in fact, turns out to be the case. A test to examine the question was carried out with commercial raw cotton samples from the 1959 crop, using fiber with DNS values of 0.83, 0.65, 0.60, 0.39, 0.30, 0.26, 0.10, 0.09, 0.07, 0.04, 0.03, 0.02. These samples were weighed to 500 mg., spread out in petri dishes, and heated in a mechanical convection oven at 160° C. for 20 minutes. It was clearly evident after heating that the samples of lowest DNS value (0.10 and below) showed very little or no browning and that the browning was definitely greater with samples of higher DNS value.

In an experiment in which raw cotton fiber was heated, the DNS value underwent a progressive decrease according to the length of the heating period. A sample which had a

TABLE 2.--Effect of humid preharvest weathering on DNS values and aqueous-extract pH of raw cotton, variety Coker 100, grown at Florence, S. C.

Year of growth	DNS values after weathering periods as follows					pH values after weathering periods as follows				
	0	1 week	2 weeks	5 weeks	10 weeks	0	1 week	2 weeks	5 weeks	10 weeks
1951.....	0.15	0.10	0.03	0.03	0.00	6.9	6.8	7.9	9.4	9.7
1952.....	.20	--	.00	.00	.00	6.8	--	8.4	9.5	8.9
1953.....	.23	.19	.11	.00	.00	7.0	7.2	8.6	8.8	8.7
1954.....	.34	.34	.28	.14	.01	6.8	6.8	6.7	8.2	7.7
1958.....	.17	.18	.10	.00	.00	6.8	6.9	7.4	8.7	9.3
1960.....	.29	--	.05	.00	.00	7.0	--	9.0	9.3	9.7

TABLE 3.--DNS values of 144 commercial cotton samples from the crop of 1959 as tested in the standard DNS procedure. Samples represent four geographical areas and four grades. (Note localization of values of 0.30 and higher (underlined) in Texas-Oklahoma and Western areas.)

Grade	Southeast area		South Central area		Texas-Oklahoma area		Western area	
	Location	DNS value	Location	DNS value	Location	DNS value	Location	DNS value
SM	Atlanta	0.13	Alexandria	0.15	Altus	0.12	Carlsbad	0.38
	Augusta	.12	Greensville	.20	Abilene	.19	El Centro	.00
	do	.04	Hayti	.20	Austin	.04	do	.25
	Birmingham	.19	Little Rock	.15	Corpus Christi	.38	El Paso	.83
	do	.22	do	.11	Dallas	.06	do	.39
	Columbia	.15	Memphis	.20	do	.15	Fresno	.28
	do	.12	do	.03	Harlingen	.37	Pecos	.28
	Montgomery	.21	do	.23	Lamesa	.12	Phoenix	.19
	Raleigh	.19	Winnsboro	.20	Oklahoma City	.27	do	.16
Average		.15		.16		.19		.31
MID	Atlanta	.09	Alexandria	.05	Abilene	.06	Carlsbad	.33
	do	.10	Blytheville	.12	do	.04	El Centro	.60
	Augusta	.23	Greensville	.14	Altus	.08	do	.15
	Birmingham	.02	do	.10	Austin	.08	El Paso	.50
	do	.07	Hayti	.11	do	.03	do	.60
	Columbia	.03	Jackson	.18	Corpus Christi	.08	Fresno	.40
	do	.16	Little Rock	.12	Harlingen	.05	Pecos	.29
	Montgomery	.11	do	.06	Lubbock	.31	Phoenix	.39
	Raleigh	.07	Memphis	.19	Oklahoma City	.00	do	.27
Average		.10		.12		.08		.39
SLM	Atlanta	.00	Alexandria	.11	Abilene	.14	Bakersfield	.25
	Augusta	.03	Blytheville	.25	Altus	.38	El Centro	.16
	Birmingham	.01	Greensville	.21	Austin	.10	do	.11
	do	.16	do	.12	Corpus Christi	.49	El Paso	.65
	Columbia	.02	Jackson	.06	Dallas	.04	do	.26
	Montgomery	.08	Little Rock	.15	Harlingen	.28	Fresno	.56
	do	.10	do	.00	Lubbock	.19	Pecos	.26
	Raleigh	.07	Memphis	.15	Oklahoma City	.30	do	.17
	do	.11	do	.14	do	.17	Phoenix	.33
Average		.06		.13		.23		.30
LM	Atlanta	.05	Alexandria	.10	Altus	.14	Bakersfield	.40
	do	.00	Blytheville	.11	Austin	.00	Carlsbad	.29
	Augusta	.00	do	.18	do	.04	El Centro	.05
	do	.00	Crenn	.17	Corpus Christi	.03	El Paso	.30
	Birmingham	.00	Hayti	.15	Dallas	.09	Fresno	.30
	Columbia	.05	do	.10	do	.03	Pecos	.33
	do	.00	Little Rock	.16	Harlingen	.14	do	.28
	Montgomery	.00	Memphis	.14	Lubbock	.39	Phoenix	.10
	Raleigh	.00	Winnsboro	.15	Oklahoma City	.04	do	.30
Average		.01		.14		.10		.26

DNS value before heating of 0.65 was heated in an oven at 100° C. for 1, 2, 4, 7, 14, and 21 days and the DNS values obtained after heating were 0.38, 0.39, 0.32, 0.29, 0.28, and 0.27, respectively.

DISCUSSION

The principal chemical constituent of a cotton fiber is cellulose, which is synthesized in the living cotton boll from sugars. When the boll opens, variable but small amounts of sugar are left in the fiber, particularly glucose and fructose. The latter are reducing sugars and cause reduction of dinitrosalicylic acid in the DNS test as here described. The response with fructose is essentially identical in a quantitative sense with that of glucose in the test. Although other intrinsic materials of a reducing nature may be present, such materials appear to be low in amount in comparison with glucose and fructose.

Spinners have been troubled recurrently by a bothersome problem of cotton fiber "sticking to the rolls," a difficulty usually ascribed to aphid honeydew on the fiber and sometimes to "high sugar cotton." Although sugar from aphid honey dew can influence the DNS value of raw cotton, the independent effect on this value of variations in the intrinsic sugar content of the fiber is so great that there is no necessary relationship between the overall reducing content of the fiber, as measured in the DNS test, and the presence or absence of aphid honeydew. Preliminary evidence suggests that ultra-violet examination supplemented by the use of a commercial test stick sold in drug stores under the name "Combistix"[4] may have value in identifying aphid honeydew deposits on cotton fiber. The latter product is a test probe for the estimation of glucose in urine, consists of a small stick impregnated with glucose oxidase and a material which reacts with H_2O_2 from the oxidase reaction to produce a color. Seed cotton known from field observation to be contaminated with aphid honeydew was provided by Mr. Glen Staten, Los Cruces, N. M. When this cotton was examined under a long-wave ultraviolet light (Blak-Ray, Model B-100, Ultra-Violet Products Inc., San Gabriel, Calif.),[4] small bluish-fluorescent granules were observed. These granules were not visible under various other fluorescent lights. When one of the Combistix test probes was wet in water and pressed against the fluorescent granules, a very dark color developed, showing a high glucose concentration in the granules. Glucose does not fluoresce but is apparently accompanied by fluorescent materials in aphid honeydew.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dorothy Nickerson of the USDA Agricultural Marketing Service, Washington, D. C., and to D. C. Harrell of the USDA Crops Research Division, at Florence, S. C., for the fiber samples used in this work.

[4] See footnote 3.

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